NSLS-II Beamline-Development Proposal Type I **X** Type II

Frontier macromolecular crystallography at an undulator beamline – FMX

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Frontier macromolecular crystallography at an undulator beamline – FMX

The mission for this project:

Macromolecular crystallography (MX) is in a Golden Age. The achievements of biologists who are able to create crystals of huge, multi-component molecular assemblies, deserve instruments to reveal structures of these assemblies. Working as a team, we represent approximately 30 scientists, engineers, and technicians who operate the nine MX beamlines at the NSLS.

- We propose to create an MX beamline that will push the state of the art in x-ray optics and sample delivery, exploiting the finest properties of NSLS-II.
- Producing a tunable beam down to one micron in dimension, with variable divergence, the beamline will address the most difficult problems in MX: small, weakly diffracting crystals, and very large unit cells.
- We will preserve beam coherence to make possible experiments we may not yet have conceived.
- We will provide cryogenic automation at the state of the art for the convenience of users.
- The station will accommodate frontier experiments to sample multiple micro-crystals in a single setting: serial crystallography.
- The beamline will begin operation at 'first light' of NSLS-II to provide continuity for ongoing NSLS user programs.

A. Science Case

Scientific Questions:

X-ray crystallography has transformed our understanding of biological processes. X-ray diffraction provided the first clues to the structure of the DNA double helix nearly 60 years ago, giving profound insights into how DNA is replicated. Driving forces for continuing development of synchrotron radiation facilities worldwide are our knowing that understanding of biological structure imparts deep insights into the mechanism of action of molecules, and that the difficulty in determining those structures increases as they get larger.

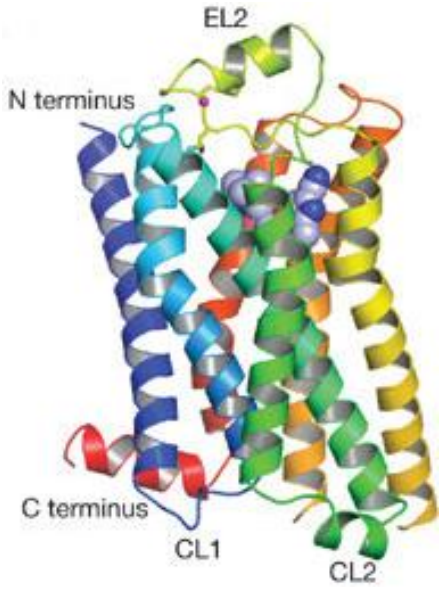
Seven recent Nobel Prize winners in Chemistry depended on readily available synchrotron X-rays for their ground-breaking research: Sir John Walker of the MRC in 1997, Roderick MacKinnon of Rockefeller University in 2003, Roger Kornberg of Stanford University in 2006, Roger Tsien of Univ. Calif. San Diego in 2008, Venkatraman Ramakrishnan of the MRC, Thomas Steitz of Yale University, and Ada Yonath of the Weizman Institute of Science in 2009. The ribosome structures, honored with the 2009 Nobel Prize, are the largest with 150,000 atoms. Determination of this structure – the location of every atom! – was an amazing *tour de force*. These awards help prove the crucial role synchrotron radiation facilities play in our understanding of the mechanisms of life.

Researchers routinely use synchrotron radiation for single-crystal diffraction studies. With the availability of brighter X-ray sources, the size and complexity of macromolecules that can be studied has increased by an order of magnitude, or three orders of magnitude in mass. Advancements observed in the past 15 years in the development of cloning, expression, purification, and crystallization methods have been impressive. However, crystals of the most complex structures that are suitable for diffraction are often scarce and difficult to obtain. Therefore, continuing advances in synchrotron radiation sources, detectors, and software are required to tackle the most challenging problems, which are the ones most likely to make a significant impact on our knowledge of the functioning of living systems.

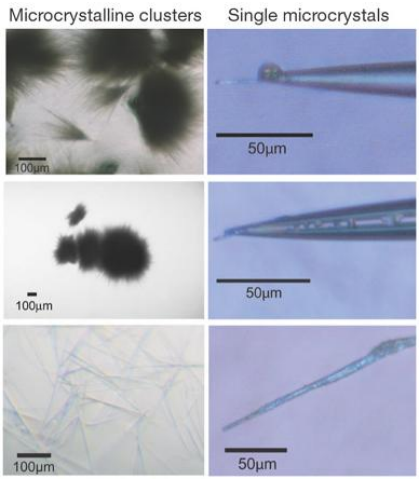
Structural biology is becoming a multi-disciplinary science. Often Small-Angle X-ray Scattering contributes information that complements crystallography. When the specimen includes a chromophore, as is often true with redox systems, coordinated diffraction and optical spectroscopy provide synergistic information. If the redox or oxygen-binding system contains a transition metal, X-ray Absorption Spectroscopy might augment crystallography. Occasionally one could improve understanding of a reaction mechanism with study of vibrational modes: Raman or infra-red spectroscopy on a single crystal. Finally, NSLS-II may improve the possibilities for synchrotron-based imaging at the several-nanometer scale in a way that would complement molecular-resolution studies. Therefore, to enrich the opportunities for such cross-cutting techniques, **we**

strongly endorse formation of a Biology Village at NSLS-II. This will put beamline staff and investigators of these disciplines together in one space. This will allow us to realize every opportunity for interdisciplinary approaches to our problems.

In recent years more MX beamlines are able to produce beams down to a few microns in size. Here are examples where availability of such a beam was critical to success. Two are the structures of the ß1 and ß2 adrenergic receptors, two members of the family of G-protein-coupled receptors. GPCRs play a major role in transmembrane signaling and many are important drug targets. Only tiny crystals were obtained of both the ß1 and ß2 receptors, and very limited crystal screening could be performed because only minute amounts of these integral membrane proteins can be produced. The structures determined by Kobilka, Stevens, Schertler and coworkers (*e.g.*, see figure, ß1 adrenergic receptor) reveal the overall architecture of the seven trans-membrane helices and associated loops, and the critical elements defining the ligand-binding pocket. These insights revolutionized the GPCR field and continue to have a dramatic impact on the development of agonists and antagonists for many members of this important family of receptors.



ß1 adrenergic receptor From: Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF. Nature 454, 486-91 (2008).



From: Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AØ, Riekel C, Eisenberg D. Nature 447, 453-7 (2007).

Needle-like micro crystals that may be 10,000 times smaller than conventional biological samples are another challenge that recently yielded structures by microdiffraction with the one micron-sized beam at ESRF's ID13. David Eisenberg and his group first succeeded in obtaining high-resolution structures from 2 µm sized crystals (see figure) containing untwisted amyloid-like fibrils, giving the first glimpse of the atomic arrangements of proteins in the amyloid state. Over the past five years, this group has determined structures for some 60 other amyloid-like microcrystals, many of which are the agents or products of nervous diseases such as Alzheimer's. Presently, working with even smaller specimens, they are demonstrating that useful diffraction can be obtained from one micron or smaller crystals.

A number of the current MX users of the NSLS responded to a request to propose work that they would do at a beamline like FMX, and to comment on how our plans would contribute to their future programs. Their letters appear in **Appendix III**. What we see is that this beamline will be in high demand. Numerous investigators have difficult membrane-protein structures in their plans, including Rod MacKinnon of Rockefeller, Fred Hughson of Princeton, Da-Neng Wang of New York Univ., Chris Miller of Brandeis, and Dax Fu of BNL. Several are doing studies on complete ribosomes or subunits, including Tom Steitz of Yale, Gerwald Jogl of Brown, and Joe Ippolito and his staff at RibX. A number are studying difficult globular RNA structures, including Anna Pyle and Scott Strobel of Yale and Alexander Serganov of Sloan Kettering. Ned Seeman continues his work on difficult-to-crystallize designed-DNA motifs. There are numerous groups studying huge complexes: Dinshaw Patel at Sloan Kettering, Yong Xiong at Yale, Huilin Li at BNL, and Todd Lowther of Wake Forest. And finally, Steve Almo and Swami Swaminathan, members of the productive New York Structural Genomics Consortium who participate in NIH's Protein-Structure Initiative, believe they will employ FMX when their project is renewed.

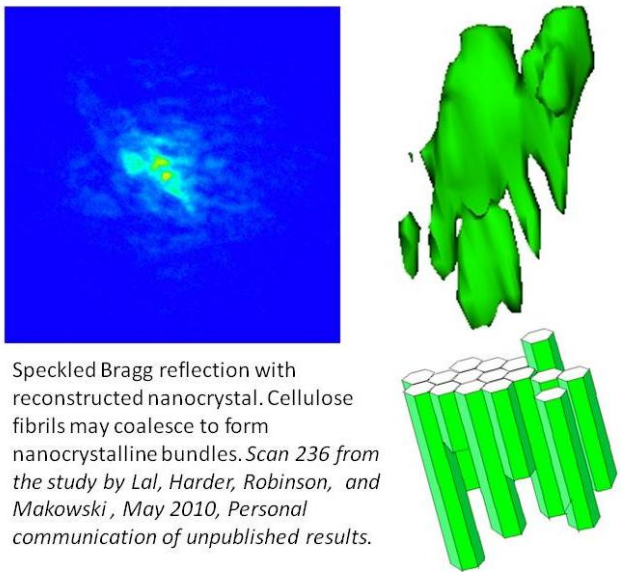
The challenges before all of our respondents – tiny crystals, poor diffraction, large unit cells – all will benefit from the brigheness of NSLS-II. They would like to exploit that brightness to provide small beam size

where necessary, and highly collimated beams where useful. **The FMX beamline we propose will accomplish this.**

Special opportunities afforded by this beamline:

Reduced radiation damage: Radiation damage in macromolecular crystals is primarily owed to energetic photo-electrons that are emitted following absorption of the beam along its path. Theoretical calculations suggest that if the beam size is sufficiently small, ≤1 μm, many photo-electrons escape the illuminated volume, thereby extending the acceptable exposure time before radiation damage results in loss of resolution in diffraction images. Experiments with beam diameters of 1 μm or more indicate that photo-electrons indeed escape the beam path, but the deduced reduction in radiation damage was less than expected. More experiments are in progress.

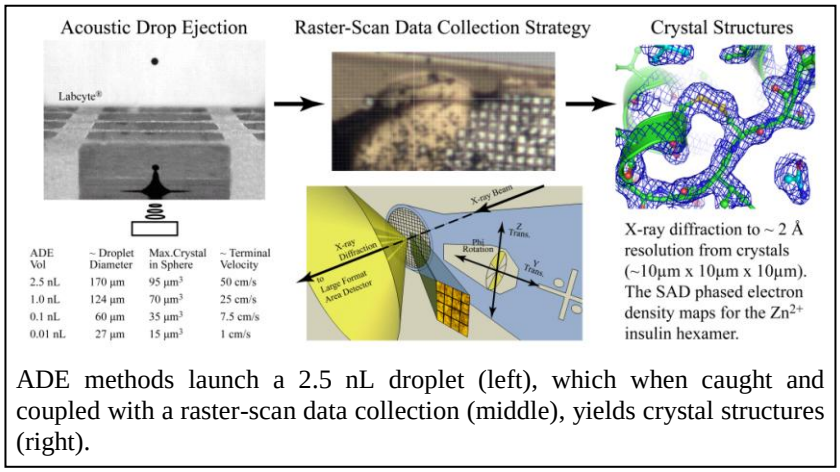
Use of beam coherence to determine nanocrystal morphology in situ: Many tissues comprise anisotropic, inhomogeneous fibrous matrices composed in part of crystalline components. These include bone, silk, amyloid, and many connective tissues. Coherent diffraction can help one to learn their structures. Recently Makowski, Lal, Harder, and Robinson, employed a hard-x-ray coherent beamline at the APS to study nanocrystals of cellulose in natural plant stems. Performing diffraction in ordinary wide-field crystallographic mode, they could distinguish individual crystallites in a fiber taken from a dried Illinois corn stalk. They identified a monoclinic form of crystalline cellulose that exhibited a range of lattice constants. Then, employing the "speckle" of the individual Bragg reflections, which represented the optical transform of the physical shape of the crystal responsible, they were able to invert these to reveal the nanocrystal morphology (see the figure). Nanocrystal reconstruction is unlikely to be a routine experiment at the FMX beamline, but this experiment, well suited to the FMX beam line, anticipates many biomedical applications and reveals a technique that overlaps imaging and crystallography.



Speckled Bragg reflection with reconstructed nanocrystal. Cellulose fibrils may coalesce to form nanocrystalline bundles. *Scan 236 from the study by Lal, Harder, Robinson, and Makowski , May 2010, Personal communication of unpublished results.*

***Microbeams afford an opportunity for serial crystallography*:** A possibility with small beams is to sample a larger crystal, either at equally spaced or pre-chosen points. This can reduce radiation damage or exploit well diffracting portions of an otherwise bad crystal. Another possibility is to assemble a data set from partial sets, each coming from one of many small crystals. To accomplish this one might employ a thin-plastic crystal-mounting grid to hold multiple small crystals, each of which may yield diffraction data. Or perhaps exposure times with the hot NSLS-II beam may be short enough that one could take partial data from micro-crystals flowing continuously in a microfluidic chamber, or somehow suspended briefly in the beam.

Acoustic droplet ejection (ADE) is a novel technology, employing focused sound waves, that we have used to transfer specimens directly from the crystallization plates onto data collection media (grids). We have already used ADE to populate a grid with a few dozen individually diffracting ~20 μm crystals. They yielded a high resolution structure when probed with a 20 μm beam at NSLS-X25 (see the figure). The tiny beam and



ADE methods launch a 2.5 nL droplet (left), which when caught and coupled with a raster-scan data collection (middle), yields crystal structures (right).

high flux expected at FMX should allow routine data collection from even smaller crystals on specimen grids prepared using ADE or similar technologies. One might imagine having a crystal-growth facility at NSLS-II where our beamline automation might complement automated crystal handling using one of these systems.

Another exciting prospect we are exploring now is an optical goniometer. It combines ADE with laser-based optical traps, as outlined in the SMX proposal. For example, a droplet/crystal is acoustically transferred into the equilibrium position of the optical trap. One might rotate the specimen within the X-ray beam, perhaps with a rotating polarized laser beam, for diffraction data collection, and then discard it when it reaches its dose limit. With this technology, this process could occur at a pace of more than one per second. Moreover, the effort and expenses of the development of this optical-goniometer / crystal-deposition system would be a collaboration within the family of MX beamlines (see the SMX proposal and the Synergy section below for more details).

Technical Capabilities:

X-Ray Source: The FMX beamline will share a pair of canted undulators with the AMX beamline in a low-β straight section of NSLS-II. The undulator will be optimized to provide the best possible performance in brightness that NSLS-II will provide, in the energy range 5-20keV, with the possibilities for some emission outside that range.

X-Ray Optics: We plan to create versatile x-ray optics for this undulator station, to allow a range of bright, tunable beams in the size range 1 - 100μm. Efforts will be made to preserve coherence of the beam for future investigations like the one described above. Probably we will focus the beam in two stages, employing a virtual source at a first-order focus point. We will choose the second-stage focusing optics from among these: bendable Kirkpatrick-Baez mirrors, either kinoform or solid-lens refractive optics, or Fresnel zone plates. However we will endeavor to build this system so that alternate focusing mechanisms may be tried or put into production with reasonable effort and expense. We'll make great effort to include ample beamline-diagnostic devices, firstly to provide the best possible performance during commissioning, and secondly to provide the option for automated realignment and optimization in the future.

Experimental Systems: The entire experimental station will provide the very best capabilities for handling of specimens down to the dimensions of 1μm. At the same time, we will provide capability for more conventional experiments with specimens in the 20 – 200μm range.

- The diffractometer will provide appropriate stability, positional accuracy, and speed while still being robust enough for routine use of a specimen automounter.
- Lighting and microscope systems will be good enough to provide visualization down to the diffraction limit of visible light.
- To provide the very best possible measurement of diffraction patterns from tiny crystals or those with very large unit cells, we will provide an area detector that is at the state of the art in these qualities, in order of decreasing priority: low noise, many pixels, small pixel size, speed at which images can be measured, and dynamic range.
- We'll employ the Python- and EPICS-based CBASS system as the overall control software for the beamline. This is absolutely the best choice for this beamline since there is local programming expertise for this system and it has evolved to meet numerous challenges over 18 years of development and revision. History has shown that these programmers respond quickly and reliably when the new apparatus is installed and commissioned. There will be no latency for startup of the beamlines. Most of the regular users of the NSLS MX facilities are familiar with and like the software.
- To allow the greatest possible flexibility in automated specimen delivery, including possibly unforeseen opportunities for introduction of diverse other crystal-manipulation apparatus, we will provide the appropriate flexibility around the specimen position.

Our goal is to be fully functioning at the very beginning, while still providing opportunities for improvements in the future.

Synergy with other similar beamlines:

The FMX beamline will be one of the six beamlines devoted to macromolecular crystallography that the NSLS MX community plans for the first few years of NSLS-II operation. Each will fill a unique niche in our overall plan to provide the best possible MX facilities for regional, national, and international scientists. And each will be part of the NSLS-II Biology Village. Here's the whole roster:

FMX	Frontier micro-focusing MX at an undulator.	Tunable 1 to 100μm beams, preservation of coherence, diverse crystal-manipulation apparatus and automated specimen delivery.
AMX	Flexible access, highly automated MX at an undulator.	Tunable 5 to 300 μm beams, automated specimen delivery, remote operation and participation, convenient access for experimenters
AM3	Flexible access MX at a 3PW.	Tunable 25 to 300μm beams, crystal screening automation, convenient access for experimenters.
SMX	Coordinated MX and optical spectroscopy at an undulator.	Tunable 5 to 300μm beams; spectroscopies to include absorption and fluorescence electronic, Raman and IR vibrational; automation for crystal mounting and integrated data collection.
SM3	Coordinated MX and optical spectroscopy at a 3PW.	Tunable 25 to 300μm beams, spectroscopies to include absorption and fluorescence electronic, Raman and IR vibrational, and XAS/EXAFS; full automated specimen handling.
NYX	NYSBC Microdiffrac-tion at an undulator	Novel high-energy-resolution monochromator produces 5-50μm beams, accurate goniometer, pixel-array detector.

The FMX beamline will complement the AMX flexible-access beamline, proposed separately. Each will have a specialized mission on an undulator source, but with significant overlap of experimental capabilities. The crystallographer who demands easy access for high quality data collection from challenging but tractable crystals will employ the AMX instrument. One who requires micro-diffraction for small or heterogeneous crystals will choose the FMX beamline. Locating both stations on a pair of canted undulators in one low-β straight section emphasizes the continuity between them: the synergy of shared access protocols, support staff, computing resources, and ancillary equipment.

Because all of these six beamlines will play a role in the comprehensive capabilities we will provide, the absence of any one will leave us with unmet needs. ***FMX is the MX beamline that will most push the high-brightness qualities of NSLS-II, so its presence is critical to achievement of NSLS-II mission goals.***

B. Beamline Concept & Feasibility

Comparing existing and planned MX micro-beamlines:

The current world-wide landscape of dedicated MX beamlines optimized to deliver small beams includes beamlines at ESRF, Diamond, SLS, and APS. Construction proceeds at PETRA-III and SPring-8 with similar plans. With the exception of ID13 at ESRF, which is used only partially for MX, none of the operational beamlines are designed to deliver a beam of 1 μm size, instead targeting a limit of about 5 μm. GM-CA/CAT at APS recently retro-fitted a beam capability of 1 μm size. GM/CA-CAT is now designing an optical system intended for a 1 μm beam. The nascent beamline at SPring-8 will deliver a 1 μm beam. Like many of the optical systems that are in place to deliver small beams, this will employ a secondary, virtual source, to provide stability and facile control of the source dimensions. We see this as an attractive possibility.

At ALBA and MAX-IV workers plan MX beamlines with a ~1 μm beam. These beamlines deserve special mention, owing to the similarity of the properties of ALBA and MAX-IV with those of NSLS-II (ring energy, emittance, etc.) MAX-IV have produced no details of their plans except the design goals below. But the ALBA proposal is already available: http://www.cells.es/Beamlines/SECOND-PHASE/MMC/proposal_mmc/. We believe this would be the first MX beamline to achieve a ~1 μm beam at the outset. It would have FWHM H/V beam size at the sample of 3 × 1 μm, with divergence 3 × 0.3 mrad. The optical scheme envisioned employs two-stage focusing in the horizontal direction and one-stage focusing in the vertical direction. The

horizontal beam size at the sample position could be doubled by removing the first horizontal focusing stage, while preserving the angular divergence. We propose something similar.

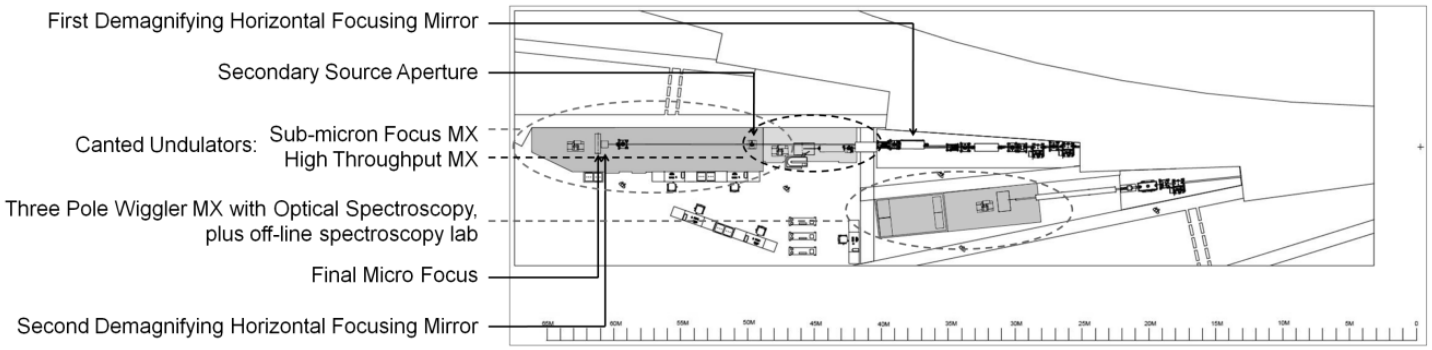
This table summarizes attributes of MX beamlines, present and future, that are designed to deliver small beams.

Beamline	*Beam Size h x v [µm x µm]*	*Flux [ph/s]*
ESRF ID13 not dedicated to MX (Ch. Riekel)	1 x 1	8×10^{10}
ESRF ID23-2 dedicated to MX, fixed λ (D. Flot)	7 x 4 (eventually 1x1)	4×10^{11}
SLS X06SA (C. Schulze-Briese)	15 x 5	10^{12}
APS GM/CA-CAT (R. Fischetti)	65 x 20 (standard) 5 x 5 (mini) 1 x 1 (micro, under re-design)	2×10^{13} 5×10^{10} 3×10^{9}
Diamond I24 (G. Evans)	9 x 9 (eventually 5x 5)	10^{12}
SPring-8 BL32XU under construction (M. Yamamoto)	1 x 1	6×10^{10}
Soleil PROXIMA 2A under construction (W. Shepard)	5 x 4 (tunable 5 – 15 keV)	1.1×10^{12}
Alba MicroFocus proposed	3 x 1	3×10^{12}
MAX IV proposed (M. Thunnissen, T. Ursby)	1 x 1	10^{13}

Concepts for a microfocusing MX beamline at NSLS-II:

We envision employing an optical system based on use of a secondary source for the FMX beamline to deliver a beam size of 1 µm. Unlike the new SPring-8 beamline design which employs one-stage focusing and places the secondary source at a different location from the focal point (thus costing significant flux), our beamline design would employ two-stage focusing in the horizontal direction and would place the secondary source at the focal point for the first horizontal focusing stage. This optimizes the flux throughput while retaining the advantages of employing a secondary source. This is similar to what is proposed for the ALBA microfocus beamline.

The figure below shows this concept, with the horizontal beam properties at points along the beamline shown in the accompanying table. We estimate that this beamline, viewing a U20 undulator source installed in an NSLS-II low-beta straight section, could deliver as much as 5×10^{11} – 1×10^{12} ph/sec at 12 keV into a 1 µm beam size with a horizontal angular divergence of 1 mrad (3 times less than being proposed for the ALBA microfocus beamline). This flux is an order of magnitude higher than has been achieved in a 1 µm beam size



Optical Component	*Location [m]*	*H-Size [µm]*	*H-Divergence [µrad]*
Source	0	66	45
Front End Slit	20	234	15
Horizontal Focusing Mirror	38	504	15 incident
Secondary Source Aperture	50	27	47
Second Horz Focusing Mirror	61	490	47 incident
Final Focal Point	61.5	≤ 1.5	≤ 1045

at the ESRF ID13 beamline or than will be achieved in a 1 µm beam size at the new SPring-8 beamline that is under construction, and comparable to what will be available at the ALBA microfocus beamline but in a less

divergent beam. (The MAX IV beam seems to be off the charts, but this ***will*** be a very bright source. The flux is quoted for 2 x 0.3mrad convergence, which is probably too big.)

In our design, one achieves a smaller focus by narrowing the Secondary Source aperture. To control divergence (convergence) is always desirable in tuning ones beam parameters to suit the specimen's requirements. We will control divergence with a slit just upstream of the monochromator. The 61.5m final distance, which underlies the conceptual layout discussed here, may increase by several meters if this beamline were located on a sector with the extended floor space made available by the recently announced introduction of bypass walkways at the building's periphery.

We desire that this FMX beamline, which would produce a ~1 μm beam, should also deliver a larger beam, up to at least 100 μm, in the same experimental station. There are several methods to accomplish this. 1) One might remove (or unbend to keep the diffractometer in the same place) the second H-focus mirror, and unbend the first H-focus mirror to move the focus from 50 to 61.5 m. We'd get a beam that is 66 μm x (61.5-38)/38 = 41 μm. This could be unbent further to get something that is as large as the full beam H opening angle. 2) If one didn't want to move the second mirror, one could increase the tilt angle on the first h-focus mirror to make it miss the second mirror, and then could readjust the first mirror as above. One would need to accommodate this with a moving diffractometer. 3) Another alternative is to provide two setups, one to deliver a 1 μm beam size and the other to deliver a larger one. Some mechanism could exchange the two, intending that the more delicate setup for the 1 μm beam is never disturbed. This approach is expected to be used at the APS GM/CA beamline where a new microfocus setup is now being designed. We will endeavor to make the change as mutually convenient and robust as possible.

C. Required Technical Advances

X-Ray optics: Mirror figure and polish must attain the desired focus. We need beam-position monitors for both the white and monochromatic beams ***that do not intersect the beam*** to disturb its coherence. Similarly we can afford only one vacuum window, and it must be optically perfect; SiN or diamond come to mind. We require a method to go flexibility from 1-100μm with the push of a button: perhaps KB mirrors that can be adaptively bent reliably with this final focal length, or kinforms that can be withdrawn reliably that will perform at this level.

Crystal visualization: With micron-sized beams, one must expect crystals of that size. We thoroughly anticipate that x-ray-based search methods will locate crystals; with quick goniometers and fast-framing, low-noise detectors, this process will take only seconds with an attenuated beam, and require minimal x-ray exposure of the specimen. However we believe that most users will want to ***see*** their crystals. We will explore use of high-quality visible light microscopes, UV illumination to produce protein fluorescence, variable and multi-direction illumination, multiple microscopes, etc. Multi-axis observation combined with computational methods may also prove useful to reconstruct the crystal scene.

Providing nano-precision goniometry: Data collection requires the rotation of crystals in the beam; probably we will employ a vertically oriented omega axis to defeat gravity. This tactic, and currently available air-bearing servo motors and screw-based translators, should get us to precision and reproducibility of fractions of a micron. Already the Crystal Logic system (Chuck Strouse's company) we have on X25 with a horizontal axis appears to allow alignment to near one micron. We will need new ideas to go much beyond this. Perhaps we might consider active feedback systems based on interferometry to true the point of interest continuously.

Providing overall thermal, vibrational, and mechanical stability: Given the smallness of a one micron beam, and the challenge we already face of performing beam alignments to several-micron accuracy, we understand that we will require superb mechanical stability. Firstly, the alignment must be ***possible***. Then it must hold up for many hours. We imagine that we might want to construct experimental apparatus on perhaps a monolithic block kept in a temperature-controlled envelope, intending to keep all of the final elements within nanometers of the line of sight. However, we are relieved to know that NSLS-II have engineers who are vibration specialists. In the end we'll get good advice from them.

Automation: Those who deal with microcrystals rarely do so by choice, and rarely does just only one such crystal yield a crystal structure. Therefore these experimenters will benefit from a high degree of automation in sample handling, mounting, and positioning, to facilitate the many trials that will be required in project development and later for data collection. This is entirely reasonable considering that current research groups already rely on the speed and steady hand of robots when screening hundreds of crystals for the few that yield good diffraction. At a microdiffraction beamline, use of robots, not humans, will be part of the method to maintain thermal stability in the hutch.

Micro-diffraction requires high-resolution detectors: Contemporary pixel-array detectors contribute no noise to x-ray measurements. However, current detectors have large pixels (150 - 170 μm square) that intercept background intensity from the specimen, its mother liquor, *etc*., over the full pixel area. By contrast, Bragg spots from micron sized beams and crystals will deposit x-rays in a fraction of that area. This will compromise their signal-to-noise ratio, and ultimately the best achievable resolution. For a high-performance microdiffraction setup we require detectors with smaller pixels, as much smaller as possible! An additional challenge will be the acquisition or development of detectors that can be used for the beam tuning and alignment process itself, to provide beam imaging, and profile and position measurements.

D. User Community and Demands

The nine existing MX beamlines at NSLS are an amazing engine for structural biology. In particular, since the beginning of 2005 there have been 2800 PDB submissions and 1795 new publications discovered from the nine active and two legacy* NSLS beamlines: X3-A, X4-A, X4-C, X6-A, X8-C*, X9-A*, X12-B, X12-C, X25, X26-C, and X29. Of the publications 185, over 10%, were in the premier journals Science, Nature, and Cell. We also observe that X29 is now the second most productive beamline in the world in PDB depositions with 250 in 2009. The highest number is 290 from the BER-funded third-generation undulator 19ID at the APS. No other beamline comes close. Three recent winners of the Nobel Prize in Chemistry, Roderick MacKinnon in 2003, and Venkatraman Ramakrishnan, and Thomas Steitz in 2009, employed beamline X25 and other NSLS beamlines for significant portions of their prize-winning work.

This remarkable productivity is possible for several reasons. An important one is that the NSLS lies within easy driving distance of the productive arc of molecular and structural biologists from Philadelphia to Boston. Many northeastern crystallographers feel they and their groups have an advantage actually to visit NSLS rather than to visit possibly more impressive beamlines in other places. Coming here affords opportunities to do the work on short notice when their needs warrant a day trip. This demand for easy, personal access will help to drive use of NSLS-II beamlines.

A second source of productivity results from the enthusiasm of the local staff for pursuing the very best apparatus and methods. During 1993 R. Sweet and L. Berman improvised data-collection apparatus to allow monochromatic MX data collection at the wiggler beamline X25 with use of an ancient Arndt-Wonacott rotation camera, individual Fuji imaging phosphors, and a Fuji BAS2000 scanner. Then X25 ran with a MAR Research automated imaging-plate scanner from 1995 until the Brandeis B4 CCD-based detector appeared in 1998. In 2002 we installed a Q315. We constructed the X29 undulator beamline by 2004, and soon implemented rapid-access protocols that led to today's multiple users on each day. We installed the X25 undulator in 2007 and have offered microbeams since 2009. Steady operation of cryogenic automounters began by about 2008. The coordinated MX and optical spectroscopy station also began operation on 2008. We will install a fast-framing Pilatus 6M pixel-array detector in 2011. **These and many continual advances have kept the attention and supported the productivity of a large user community**.

A third and critical reason for the high productivity of NSLS MX beamlines is the strong culture of service within the beamline staff. We pioneered the use of the Web for rapid-access proposals back when the only web browser was "Mosaic" (the early '90s); now essentially all of the beamlines are accessible in this way. A comprehensive experiment-tracking database system is coupled to the proposal process. Also available is the transformative and hugely productive Mail-In crystallography program for academic users. Finally we offer a regular series of training opportunities, including crystal-growth workshops, the Crystallographer's Workbench,

and the internationally renowned RapiData course, now with nearly 600 alumni. **This emphasis on the investigators and their needs keeps a focus on the NSLS MX beamlines and their capabilities.**

A fourth strength of MX programs at the NSLS is the frequent interaction between the greater MX community and our resident staff. In addition to contacts at beamlines, meetings, and courses, all of which yield new ideas and countless suggestions, we actively recruit the MX community to help in long-range planning. These investigators helped to make the original scientific case for NSLS-II, and area crystallographers helped drive the many workshops that underpin this proposal. We created a chronology of our planning for MX at NSLS-II in **Appendix II**, including mention of our interaction with the local community. We seek, and then employ, advice and assistance from our users.

The particular characteristics of the **FMX** beamline, to push to new frontiers in beam size, collimation, and sample delivery, will likely benefit all of these investigators at some time. Please find letters **Appendix III** from the many scientists who have written to describe work they are planning that will require FMX.

The remainder of the proposal, containing personal and financial information, is removed.